

Space Sciences Laboratory
University of California
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Semi-Annual Report on
REFLECTION SPECTRA AS A BASIS FOR
STUDYING EXTRATERRESTRIAL LIFE

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PLANETARY SPECTROSCOPY

Progress on this grant has been somewhat limited due to the preoccupation with the infrared telescope site evaluation.

Accomplishments During the Past Six Months and Current Status

1. Construction of the photometer-polarimeter has continued, although somewhat haltingly. It is anticipated that it will be completed shortly and that results will flow from it rapidly since Brian O'Leary, whose thesis includes this work, hopes to finish in September.

2. Progress on the simulation of weathering phenomena on Mars has been notable since February due to the arrival of Dr. J. T. O'Connor, formerly of the U. S. Geological Survey, Astrogeology Branch, Flagstaff. All of the components of the high vacuum system have been ordered and many of them received, and it should be in operation in the very near future. This work is primarily funded by NASA Contract NASr 220.

3. Rea and O'Leary have carried out a careful examination of the evidence reportedly indicating H_2O ice clouds on Venus. They concluded that the evidence in fact indicates exactly the opposite.

4. Welch and Rea have utilized recent determinations of the HCl mixing ratio on Venus in conjunction with microwave data to set an upper limit on the amount of liquid water in the Venusian atmosphere. Their value of 0.010 g cm^{-2} corresponds to a cloud thickness of only 100 m for a water concentration of 1 g m^{-3} , typical of terrestrial clouds. This low upper limit has a significant bearing on interpretations of the microwave data, on greenhouse effect calculations, and on the possibility that the upper clouds are H_2O ice.

5. Calculations on the effects of topography on the deposition and lifetime of solid CO_2 on the Martian surface indicated that the Mountains of Mitchel are, in fact, most probably depressions 6–9 km lower than their surroundings. Since, when the deposits are not present the Martian surface at these positions is bright, this supports previous work from this laboratory suggesting that the bright areas are depressed and the dark areas elevated.

Proposed Program for the Next Six Months

1. Construction of the photometer-polarimeter will be completed and will be used to acquire data on samples of Martian interest.

2. The Martian weathering simulator will be completed and will be put into operation.

3. Observations of Venus will be carried out to detect the possible presence of chlorine gas in its upper atmosphere. Due to the recent discovery of HCl on Venus, Cl_2 may exist in the upper atmosphere where O_2 is produced by photodissociation of CO_2 . If it does, it may prove to be the source of the yellow tint of the planet.

Publications and Lectures

O'Leary, B. T., "The Presence of Ice in the Venus Atmosphere as Inferred from a Halo Effect," *ASTROPHYSICAL JOURNAL*, 146, 754 (1966).

O'Leary, B. T., and Rea, D. G., "Mars: Influence of Topography on Formation of Temporary Bright Patches," *SCIENCE*, 155, 317 (1967).

Welch, W. J., and Rea, D. G., "Liquid Water in the Venus Atmosphere," *ASTROPHYSICAL JOURNAL* (in press).

Rea, D. G., and O'Leary, B. T., "On the Composition of the Venus Clouds," *JOURNAL OF GEOPHYSICAL RESEARCH* (submitted for publication).

Buhl, David, "Radiation Anomalies on the Lunar Surface," Ph. D. Thesis and Technical Report, Space Sciences Laboratory Series No. 8, Issue No. 1 (January, 1967).

Rea, D. G., and O'Leary, B. T., "On the Composition of the Venus Clouds," presented by D. G. Rea at the 123rd Meeting of the American Astronomical Society, December 27-30, 1966, Los Angeles, California.

Rea, D. G., "Water Clouds on Venus?" Seminar presented at the Jet Propulsion Laboratory, Pasadena, California, on March 31, 1967.

Personnel

Dr. Donald G. Rea — Project Director; Roger Dorr — Specialist; Michael Graham — Research Assistant; Dr. Joseph T. O'Connor — Assistant Research Geologist; Brian O'Leary — Graduate Fellow; Malcolm Raff — Research Assistant; John Sexton — Senior Engineering Aid; David Wilson — Junior Development Engineer; and Dawn Nyman — Principal Clerk.

Electronics technicians and machinists have also been employed at various stages of the work.

GEOCHEMISTRY AND MASS SPECTROMETRY

This report presents a comprehensive summary of the geochemical and mass spectrometric studies that have been initiated, continued, or completed during this semi-annual period.

The geochemical results derive from work by a group under the supervision of Professors Melvin Calvin and Alma L. Burlingame at Berkeley and from work by Dr. Geoffrey Eglinton's group in Glasgow, Scotland. The mass spectrometry research has been carried out under the direction of Professor Burlingame in the Space Sciences Laboratory.

I. GEOCHEMISTRY

A. Berkeley Group

1. Organic Constituents of Mud Lake, Florida. Mud Lake, Florida is considered to be a modern environment of the same type that may have resulted in the deposition of ancient non-marine oil shales such as the Green River Formation. An analysis of the organic constituents in the deposited mud layers of various depths, as well as of contemporary organisms (such as algae) living in this environment, might yield important data on the diagenesis of organic compounds.

A heptane extract of the dried and pulverized mud (after silicic acid chromatography and separation of one of the fractions on thin-layer plates) yielded a mixture of saturated hydrocarbons in which the $n\text{-C}_{27}$ and $n\text{-C}_{29}$ compounds are major components; another fraction contained β -carotene. The latter substance was identified by comparison of its visible and mass spectra with those of authentic β -carotene. After similar fractionation, the carotenoid xanthophyll can be isolated from the methanol extract of the mud sample. Analyses of several other mud layers have provided tentative evidence for the presence of pheophytin a and rhodoxanthin.

2. Porphyryns. A program aimed at the isolation and identification of the porphyrin constituents of ancient sediments has been started. Since gas chromatography appears essential for the isolation of individual porphyrins, initial work was directed toward the preparation of volatile porphyrin derivatives. Several silicon etioporphyrins have been prepared, and preliminary gas-liquid chromatography (g.l.c.) tests with these compounds have given encouraging results. Current studies are concerned with the improvement of the synthetic procedures, the preparation of several other types of silicon porphyrins, and the elucidation of their structures by a combination of visible spectroscopy and high and low resolution mass spectrometry.

3. Green River Shale

a. Triterpanes; Steranes. The analysis of the sterane-triterpane fraction of the Green River Shale extract is continuing. A relatively large quantity of pulverized rock (~ 15 kg) was extracted, and from the total extract ($\sim 1.7\%$ weight of rock) a hexane fraction containing saturated hydrocarbons was obtained by column chromatography on alumina. This hydrocarbon fraction (after removal of the normals by sieving) yielded (after further column chromatography, preparative g.l.c., and sublimation) several pure fractions of steranes and triterpanes. Several new triterpenoid substances have been isolated. The optical rotations have been determined for some triterpane fractions; these were found to correspond to the values expected for biologically synthesized triterpanes.

b. Acids. The acidic fraction obtained from the total extract by treatment with sodium hydroxide yielded (after formation of the methyl esters) about forty components, which were separated and purified by g.l.c. and were analyzed by low and high resolution mass spectrometry. A series of straight chain dicarboxyl acids, aromatic acids, and saturated cyclic acids have been identified.

c. Bases. The fractionation of basic components isolated from the Green River Shale by means of column, thin-layer, and gas chromatography is in progress. A high resolution mass spectral study of several base fractions has permitted the recognition of various structural types, among which the substituted pyridines and cycloalkyl pyridines appear to predominate.

d. Kerogens. The insoluble kerogen fraction of ancient sediments constitutes the major portion of all organic matter present in the rock. Therefore, an investigation into the nature of kerogen is of considerable importance in relation to the diagenetic transformation of organic compounds. In our experiments, kerogen—the polymeric material obtained from the shale after dissolution of the rock matrix by hydrofluoric acid—was degraded by oxidation in chromic acid solution. The acids thus obtained were converted to their methyl esters, were separated into normal and branched esters by urea adduction, and were analyzed by gas chromatography and mass spectrometry. Preliminary results indicate the presence of a series of normal acids, as well as several branched compounds, one of which exhibits the mass spectral fragmentation pattern and g.l.c. retention time of phytanic acid methyl ester. The identification of other acids isolated from the kerogen oxidation, as well as experiments with other degradative methods, is in progress.

4. Irradiation and Pyrolysis Studies. A study is in progress of the products formed upon electron irradiation (5 MeV) of solid methane. Initial results indicate the formation of small amounts of high molecular weight products (up to C_{25}), but no preference for the synthesis of any specific compound is apparent.

Since hydrocarbons of the isoprenoid type isolated from sediments could be derived from such precursors as squalane, the products formed upon pyrolytic degradation of squalane are under

1. Fatty Acids from Sediments. The project involves (a) the isolation and identification of fatty acids from the Green River Shale, Torbonite, and for comparison the living alga Botryococcus braunii Kutzing A, and (b) the development of techniques to isolate and identify micro-quantities of acids.

From the Green River Shale an acid fraction was isolated by column chromatography after demineralization of the rock, hydrolysis, and extraction. After methylation and subsequent thin-layer chromatography, the ester fractions were examined by g.l.c. and g.l.c.-mass spectrometry. Mono-carboxylic acids ranging from C_{13} to C_{32} were found, as well as the isoprenoid acids from C_{14} to C_{17} and from C_{19} to C_{21} . The C_{28} normal acid and the C_{20} isoprenoid acid are the dominant components. The yield of total fatty acids was 0.035 % of the non-demineralized shale.

The Torbonite, a carboniferous deposit that is presumed to contain as part of its organic matter the remains of the living alga Botryococcus braunii has been similarly analyzed. A yield of 0.007% of total fatty acid was obtained, of which the normal acids from C_9-C_{21} and α - ω dicarboxylic acids from C_8-C_{22} have been identified thus far.

Botryococcus braunii contains 0.014 % total fatty acids, among which a normal C_{18} monosaturated acid predominates, followed by the n- C_{16} and n- C_{28} acids. Dicarboxylic acids ($C_{14}-C_{19}$) could also be identified. The normal acids, obtained after hydrogenation of the total ester mixture, range from $C_{14}-C_{20}$, with C_{18} , C_{16} , and C_{28} predominating. Small quantities of branched acids are also present.

2. Determination of Absolute Configuration of Organic Compounds from Geological Sources. The possibility of micro-scale determination of the absolute configuration of branched chain fatty acids and related compounds is being investigated. It is hoped that preparation of diastereomeric esters will allow separation of enantiomers by capillary g.l.c. and, with gas chromatography-mass spectrometry for definition of gross structure, will provide a means of correlating the stereochemistry of these compounds.

At present, the feasibility of this project is being examined using compounds derived from phytol, which contain two asymmetric centers known to be of the R configuration. The studies involve (a) reduction of phytol (generating another asymmetric center, which is then both R and S), oxidation to phytanic acid, and esterification with an optically active alcohol to generate the diastereomeric esters; (b) the formation of pristanic acid from phyt-1-ene; and (c) the esterification of dihydrophytol (derived from phytol) with S(+) lactic acid to give again a mixture of diastereomers.

As yet, a resolution of such diastereomeric esters on g.l.c. columns has not been achieved due to the inadequacy of the columns at hand.

3. Cutin Acids. In preparation for the analysis of fossil cutin (the outer protective layer of fruits and leaves), the fatty acids of apple cutin were analyzed. After preparation of the cutin, hydrolysis of the polymeric acid material (methanolic potassium hydroxide), and preparation of the methyl esters of the ether-soluble acids, the ester mixture was fractionated by thin-layer chromatography (silica gel) and the components of each fraction were analyzed. So far, gas chromatography, mass spectrometry, IR, and comparison of the R_f values with known acids permitted the identification of a series of normal fatty acids, three diacids (C_{16} , C_{18} , octadec-en-1, 18-dioate), and a number of hydroxy acids (C_{18} -18-OH, C_{16} -16-OH, C_{18} -10-OH, C_{18} -9, 10, 18-tri-OH, C_{16} -10, 16-di-OH), among which methyl 10-hydroxyoctadecanoate, methyl octadecan-1, 18-dioate, and methyl octadec-(?)-en-1, 18-dioate previously had not been reported from cutin.

4. Clathration Experiments. Conditions have been worked out for the separation of micro-quantities of branched or cyclic alkanes from complex mixtures by thiourea adduction. In model experiments with alkanes from the Green River Shale, it was demonstrated that branched-cyclic isoprenoids, as well as the steranes cholestane, ergostane, and sitostane, are adducted by thiourea. Studies are in progress to elucidate the nature of this adduction process. The non-adducted part of the hydrocarbon mixture (from which n-alkanes

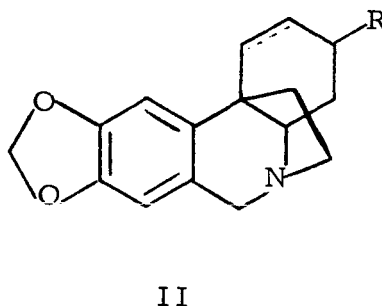
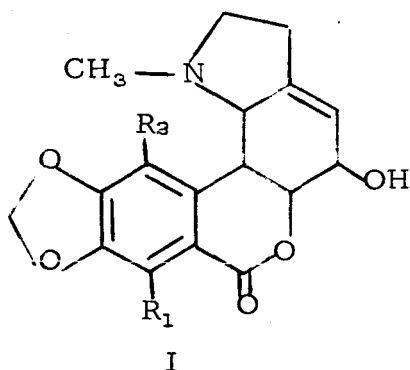
previously had been removed) consisted largely of pentacyclic triterpanes, but contained also the C₄₀ isoprenoid β -carotane—not previously isolated from this shale.

II. HIGH RESOLUTION MASS SPECTROMETRY

A. Mechanistic Studies

1. Amaryllidaceae Alkaloids. A comprehensive investigation of the fragmentation processes of Amaryllidaceae alkaloids in the mass spectrometer has been undertaken and is largely completed. Using mainly high resolution mass spectrometry, several classes of these alkaloids were studied concurrently.

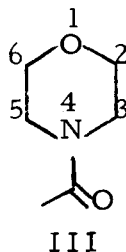
a. Lycorenine Alkaloids. A study of the alkaloids of the lycorenine type (mentioned in the last semi-annual report) has been completed. The major fragmentation pathway of these compounds (Structure I) involves a retro Diels-Alder fragmentation producing an ion corresponding to the aromatic moiety, as well as the very abundant nitrogen-containing fragment. The study of a variety of compounds of this class showed that the spectra of these alkaloids permit the identification and location of substituents in various parts of the molecules.



b. The Crinine Class. The Amaryllidaceae alkaloids of general Structure II (crinine series) have received detailed attention. A very extensive collection of these compounds (possessing functionalities or substituents at almost every carbon in II), as well as related model compounds, have been studied by high resolution mass spectrometry, permitting a detailed mechanistic description of the fragmentation processes (such as, for instance, the elimination of nitrogen-containing fragments). This study bears particular relevance to future structural

work in this series. Furthermore, some interpretations of the mass spectrometry of these systems published by other groups have been shown to be incorrect.

2. N-Acetyl-Morpholine. Fragmentation of N-acetyl-morpholine (III) in the mass spectrometer has been studied in detail. The mass spectrum of this compound with peaks at m/e 129 (M^+), 114, 86, 56, 57, and 43 is particularly intriguing since the elimination of 15 m.u. from the molecular ion constitutes an important fragmentation process, even though no methyl groupings (aside from the acetyl-methyl) are available. The combination of deuterium labeling of all positions and high resolution mass spectrometry permitted the elucidation of all important fragmentation sequences and also established that the methyl group eliminated derived from the 3-position of the molecule. The extra hydrogen radical required for this elimination was shown to come from C-2.



3. α - β Unsaturated Ketones. Substituted cyclic hexenones possessing an α, β double bond undergo fragmentation with elimination of ketene (CH_2CO). Since this process involves cleavage of a vinylic bond—an unusual fragmentation—the mechanism of this reaction was investigated in some detail. Particular attention was given to the question of the relationship of the mass spectral decomposition to the known photolytic rearrangements of such systems. In analogy to photochemical transformations, the involvement of a cyclopropane intermediate in the mass spectrometric elimination of ketene was suggested. The generality of this parallel between photochemical reactions and mass spectrometric fragmentation has been studied, as well as the dependence of the fragmentation process on substitution pattern. Results indicate that the photochemistry-mass spectrometry similarity may only apply in very few situations. In conjunction with this work, a variety of cyclic ketones and photoproducts derived from them have been studied.

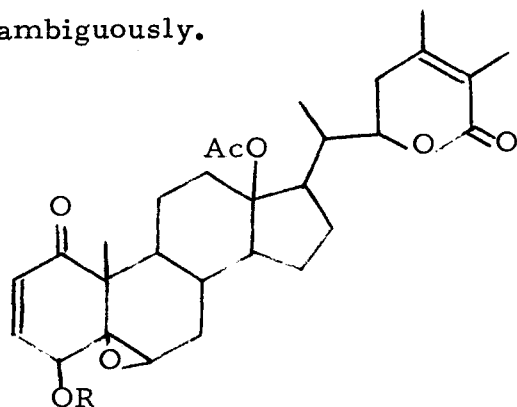
4. Cycloheptenones. A mass spectrometric investigation of the behavior of cycloheptenones (α,β and β,γ) has been started to gain some insight into the complex fragmentation processes of natural products possessing this structural moiety.

5. Flavones and Coumarins. An extensive study of the fragmentation pattern of heteroatomic aromatic systems has been undertaken. Particular attention was directed to oxygen heterocycles such as flavones and coumarins. An investigation by high resolution mass spectrometry permitted a fairly complete analysis of the fragmentation of these compounds.

6. Lycopodium Alkaloids. A study of the mechanism of decomposition of alkaloids of the Lycopodium series has been in progress in our laboratories for some time and is now essentially complete. High resolution mass spectrometry permitted a detailed delineation of mechanistic pathways, which in turn were found to be characteristic for each skeletal type and its substitution pattern. Information obtained from these known systems can now be applied to new and unknown structures in this series.

B. Application to Structure Elucidation of Natural Products

1. Withaferin-A Series; Other Natural Products. High resolution mass spectrometry permitted the tentative assignment of Structures IV and V to two new compounds of the withaferin series. Further high resolution studies are in progress to establish these structures unambiguously.



IV R = H

V R = Ac

2. Structural studies on several new compounds belonging to the sesquiterpene and steroid classes are in progress. The structure of a new nitrogen-containing tumor inhibitor is under investigation.

III. PROPOSED PROJECTS FOR THE ENSUING PERIOD

The individual geochemistry projects mentioned in this report will be continued. Particular attention will be paid to the development of new techniques for the isolation and characterization of microgram samples of individual compounds. The program is to be expanded to the study of additional geological materials (shales, oils, fossils, etc.) and other classes of compounds derived from them.

Our efforts in mass spectrometry will continue to embrace the following major areas:

- (1) the study of the mechanism of electron-impact-induced fragmentation of particular classes of compounds and, associated with this, the application of computer methods to the interpretation of high resolution mass spectra;
- (2) the elucidation of structures of natural products, particularly those of biological interest.

IV. PERSONNEL

Space Sciences Laboratory, Berkeley:

Under the direction of Professors M. Calvin and A. L. Burlingame the following personnel have been engaged in the projects described in this report: D. B. Boylan — Post Graduate Research Chemist; C. F. Fenselau — Post Graduate Research Chemist; J. Han — Teaching Assistant; P. Haug — Research Assistant; W. Van Hoeven — Research Assistant; P. Longevialle — Assistant Research Chemist, NATO Fellow; E. D. McCarthy — Research Assistant; H. K. Schnoes — Assistant Research Chemist; B. Simoneit — Spectroscopist; D. H. Smith — Assistant Research Chemist; E. Sloan — Laboratory Technician; J. Tesarek — Research Assistant; F. Walls — Spectroscopist; P. Murphy — Laboratory Technician; and S. Noton — Secretary-Stenographer.

University of Glasgow, Scotland:

The Glasgow Group, under the direction of Dr. Geoffrey Eglinton, includes the following personnel: Dr. K. Douraghi-Zadeh, Dr. I. Maclean, Dr. J. Maxwell, and Sister M. T. J. Murphy — Research Associates; D. H. Hunneman — Graduate Student; and I. McGeachie — Secretarial Assistant.

V. PUBLICATIONS: The following papers have been published in the period since the last report:

Johns, R. B., T. Belsky, E. D. McCarthy, A. L. Burlingame, P. Haug, H. K. Schnoes, W. Richter, and M. Calvin. The Organic Geochemistry of Ancient Sediments, Part II. *Geochim. Cosmochim. Acta* 30, 1191 (1966).

Van Hoesen, W. H., P. Haug, A. L. Burlingame, and M. Calvin. Hydrocarbons from Australian Oil, Two Hundred Million Years Old. *Nature*, 211, 1361 (1966).

Bryant, W. H., III, A. L. Burlingame, H. O. House, C. G. Pitt, and B. A. Tefertiller. The Mass Spectra of Derivatives of 3-Azabicyclo(3.2.1)octane, 3-Azabicyclo(3.3.1)nonane, and 8-Azabicyclo(4.3.1)decane. *J. Org. Chem.*, 31, 3120 (1966).

Fenselau, C. F., W. Richter, and A. L. Burlingame. High Resolution Mass Spectrometry in Molecular Structure Studies: Widdrol. *J. Am. Chem. Soc.* (in press).

Kupchan, S. M., J. M. Cassady, J. E. Kelsey, H. K. Schnoes, D. H. Smith, and A. L. Burlingame. The Structure of Gaillardin. *J. Am. Chem. Soc.*, 88, 5292 (1966).

Döpke, W., M. Bienert, A. L. Burlingame, H. K. Schnoes, P. W. Jeffs, and D. S. Farrier. Clivonine and Clivimine. *Tetrahedron Letters*, No. 5, 451 (1967).

Eglinton, G., and M. Calvin. Chemical Fossils. *Scientific American*, 216, 32, (1967).

Eglinton, G., A. G. Douglas, J. Maxwell, J. N. Ramsay, and S. Stallberg-Stenhagen. Occurrence of Isoprenoid Fatty Acids in the Green River Shale. *Science*, 153, 1133 (1966).

Smith, D. H. High Resolution Mass Spectrometry: Techniques and Applications to Molecular Structure Problems. Doctoral Dissertation, Department of Chemistry, UCB, March, 1967.

Maxwell, J. Doctoral Dissertation, University of Glasgow, 1967.

VI. TALKS

Douglas, A. G., K. Douraghi-Zadeh, G. Eglinton, J. R. Maxwell, and J. N. Ramsay. Fatty Acids in Sediments Including the Green River Shale (Eocene). Presented at the 3rd International Meeting on Organic Geochemistry, London, Sept. 1966.

Douglas, A. G., G. Eglinton, and W. Henderson. Thermal Alteration of the Organic Matter in Sediments. Presented at the 3rd International Meeting on Organic Geochemistry, London, Sept. 1966.

Eglinton, G., J. R. Maxwell, M. T. J. Murphy, W. Henderson, and K. Douraghi-Zadeh. Hydrocarbons and Fatty Acids in Algal Shales and Related Materials. Presented at the Meeting of the Geological Society of America, San Francisco, Nov. 14-16, 1966.

Burlingame, A. L. The Organic Geochemistry of Ancient Sediments. Presented at the Meeting of the Geological Society of America, San Francisco, Nov. 14-16, 1966.